

US

# Anti-Human Globulin

## Anti-IgG, -C3d; Polyspecific

(Rabbit/ Murine Monoclonal)

FOR IN-VITRO DIAGNOSTIC USE

For Tube Testing

MEETS FDA POTENCY REQUIREMENTS

U.S. License Number: 1798

### Package size

[REF] 804115100 [VOL] 10 x 10 mL Anti-Human Globulin, Anti-IgG, -C3d; Polyspecific

### Intended Use

Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is used for the direct antiglobulin test to demonstrate the in-vivo coating of red blood cells with antibody molecules and/or complement components (such as autoantibodies, maternal antibodies in hemolytic disease of the newborn, alloantibodies against red blood cells in transfusion reactions).

Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is used for the indirect antiglobulin test to demonstrate the in-vitro coating of red blood cells with antibody molecules and/or complement components as in detection and identification of unexpected antibodies as well as crossmatch tests. Furthermore, blood group antigen typing (with the corresponding test reagent for the indirect antiglobulin-test) can be carried out.

### Summary

Moreschi first described the use of Anti-Human Globulin in 1908<sup>1</sup>. Coombs rediscovered the test in 1945.<sup>2,3</sup> By injecting rabbits with human IgG, they were able to produce a protein (Anti-IgG) that reacted with "incomplete" antibodies (IgG). Most "incomplete" antibodies (IgG) fail to agglutinate red blood cells suspended in saline.<sup>4</sup> Most clinically significant antibodies in red blood cell serology are of the IgG class and can only be detected by the use of Anti-IgG. A stable lattice structure is formed and agglutination occurs when Anti-IgG binds to the IgG sensitized red blood cells.

Biotest Anti-Human Globulin reagents are used to test for the presence or absence of unexpected red blood cell antibodies. Furthermore, blood group antigen typing (with the corresponding test reagent for the indirect antiglobulin test) can be carried out. Routine pretransfusion studies always include tests for antibody screening, crossmatch and antibody identification.

### Principle of the Test

The test principle is a hemagglutination test. Anti-Human Globulin Anti-IgG, -C3d; polyspecific acts as a link between the antibody and/or complement coating of neighbouring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate.

### Reagent

Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is a blend of rabbit anti-IgG and murine monoclonal anti-complement (murine IgM Anti-C3d, Bric 8). The anti-IgG component contains antibody reactivity against light IgG chains and thus may also agglutinate IgA or IgM coated red blood cells. The anti-complement component consists of murine monoclonal IgM anti-C3d-antibody reactive with C3b- and C3d-coated red blood cells.

Antibodies are diluted in a isotonic saline solution containing bovine albumin and as colorant Patent Blue and Tartrazin.

The following antibodies are produced using intermediate products produced for Biotest Medical Diagnostics GmbH in a shared manufacturing agreement with Millipore (UK) Ltd., 9 Fleming Road, Kirkton Campus, EH547BN, Livingston, UK; License Number 1721.

Anti-C3d                      clone BRIC 8 (IgM)

Preservative: 0.1% sodium azide.

### Precautions

- For In-vitro diagnostic use.
- Store at 2 to 8°C.

- Do not use beyond the expiration date.
- Do not use if turbid.
- Do not dilute.
- Do not use specimens collected with gel separators.
- Handle and dispose of reagents as potentially infectious
- Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
- Caution: This product contains Natural Rubber Latex Which May Cause Allergic Reactions.
- Warning: Contains sodium azide (NaN<sub>3</sub>), which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the build-up of explosive metal azides.
- The bovine albumin used for the production of this reagent is purchased from BSE-free US sources, Boval Company L.P. in Cleburne, Tx, USA and Millipore in Kankakee, IL, USA.

### Specimen Collection

Fresh samples of clotted or EDTA anticoagulated whole blood can be used for the indirect antiglobulin test. EDTA or citrate anticoagulated whole blood samples must be used for the direct antiglobulin test, weak D test or crossmatch. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C. Stored samples should be allowed to reach room temperature prior to testing. Use of samples older than ten days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples. Blood specimens exhibiting gross hemolysis or contamination should not be used.

### Materials

#### Materials provided

- Anti-Human Globulin Anti-IgG, -C3d; Polyspecific

#### Material required but not provided

- Pipettes (drop volume 40 to 50 µL)
- Isotonic saline solution
- Reagent red blood cells (e.g. Biotest: Biotestcell® 1 & 2 [REF] 816014100, Biotestcell® 3 [REF] 816085100, Biotestcell®-I 8 [REF] 816020100, Biotestcell®-I 11 [REF] 816021100)
- Donor or patient red blood cells
- IgG coated red blood cells (e.g. Biotest Coombscell-E [REF] 816030100)
- LISS (e.g. Biotest MLB2 [REF] 805200100)
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological Centrifuge
- Interval Timer
- Markers
- Optical aid (optional). The use of an optical aid for agglutination reading must be validated by the user.

### Test Procedure

#### A. Indirect Antiglobulin Test (IAT)

If an enhancement medium (albumin, LISS) is used, please refer to the respective instructions for use.

1. Prepare a 3 to 5 % suspension of red blood cells in isotonic saline solution.
2. Place 1 drop of red blood cell suspension in an appropriately marked tube and add two drops of serum to be tested (or as directed for test reagent).
3. Incubate at 37°C for 30 to 60 minutes or as appropriate to the enhancement reagent used.
4. Wash the red blood cells 3 times with isotonic saline. Decant supernatant saline completely.
5. Add 2 drops of Anti-Human Globulin Anti-IgG, C3d; Polyspecific to the packed red blood cells and mix.
6. Centrifuge for 20 seconds at 800 - 1000 x g.
7. Gently dislodge the red blood cell button and observe for agglutination.
8. Record results

#### B. Direct Antiglobulin Test (DAT)

**FOR REFERENCE USE ONLY: DO NOT USE in place of package inserts provided with each product.**

1. Prepare a 3 to 5 % suspension of the red blood cells in isotonic saline.
2. Wash 1 drop of this red blood cell suspension 3 times, with isotonic saline. Decant supernatant saline completely.
3. Add 2 drops of Anti-Human Globulin Anti-IgG, C3d; Polyspecific to the packed red blood cells and mix.
4. Centrifuge for 20 seconds at 800 - 1000 x g.
5. Gently dislodge the red blood cell button and observe for agglutination.
6. Record results

#### Stability of the Reaction

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

#### Quality Control

The reactivity of all reagents should be confirmed by testing with known positive and negative red blood cells on each day of use.

To confirm the reactivity or specificity of Biotest Anti-Human Globulin Anti-IgG, -C3d; Polyspecific, the reagent should be tested with IgG coated (and if possible complement coated) and non coated red blood cells respectively. The reagent is satisfactory for use if it reacts only with the IgG (and complement) coated red blood cells.

Negative results in an antiglobulin test should be verified with IgG coated red blood cells: Add 1 drop of IgG coated red blood cells, mix and centrifuge for 20 seconds at 800 - 1000 x g.

Positive result: The negative reaction in the indirect antiglobulin test is valid, reactive Anti-Human globulin is present.

Negative result: A technical error was made and the test must be repeated.

It is recommended that a positive and a negative control be performed in parallel with testing.

#### Interpretation of results

Agglutination of the red blood cells with the indirect antiglobulin test is a positive result and indicates the presence of an unexpected antibody(ies). Agglutination of the red blood cells with the direct antiglobulin test is a positive result and indicates an auto-agglutinin or auto antibodies. No agglutination is a negative result and indicates the absence of an unexpected antibody or the absence of the corresponding antigen or lack of an auto-agglutinin.

An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual, 15th edition).

#### Limitations

- Low frequency antigens may not always be present on reagent red blood cells and a double dose of antigen may be required to detect very weakly reacting antibodies. Therefore, negative reactions with the screening red blood cells do not always indicate the absence of unexpected antibodies.
- Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-Human Globulin Anti-IgG, -C3d; Polyspecific.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Antibodies to antibiotics or other reagents
  - Cold Antibodies
- Positive reactions may be seen from individuals who have received Rh Immunoglobulin.
- Negative reactions will be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red cell antibodies.
- **Do not use frozen/deglycerolized and enzyme treated red blood cells**

#### Specific Performance Characteristics

Testing is performed in accordance with FDA recommended methods. The final release testing is performed according to the product specific SOPs. Each lot of Biotest Anti- Human Globulin reagent is tested in the Quality control by package insert method against IgG and complement

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coated red blood cells to insure suitable reactivity. The products meet FDA potency requirements. The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs.

For the product performance it is necessary to adhere to the recommended method in the instructions for use.

The performance of the Biotest Anti-Human Globulin Anti-IgG, -C3d; Polyspecific was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

For Technical Support or further product information, contact Biotest Diagnostics Corporation at 800-522-0090.

#### Note

Each facility should verify the optimum spin time for the specific centrifuge in use.

Manual techniques are to be performed according to the manufacturer's instructions. Each deviation from these instructions is the sole responsibility of the user. Used tests must be discarded as hazardous material. Manage waste according to national regulations.

#### Glossary of Symbols

Symbol	Definition	Symbol	Definition
<u>LOT</u>	Batch Code	<u>IVD</u>	<i>In vitro</i> diagnostic medical device
⚠	Caution, consult accompanying documents	📖	Consult instructions for use.
🏭	Manufacturer	🕒	Use by YYYY-MM-DD
⚠	Contains sufficient quantity for <n> tests.	<u>REF</u>	Catalog number
🌡	Temperature limitation	<u>VOL</u>	Volume

#### Bibliography

1. Moreschi C. Neue Tatsache über die Blutkörperchen Agglutinationen, Zbl Bakt 1908; 46:49,456
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3. Coombs, RRA, Mourant AE and Race, RR: "In vivo isosensitization of red blood cells in babies with hemolytic disease." Lancet i: 264, 1946
4. Pittiglio, D. Harmening. Modern Blood Banking and Transfusion Practices. Philadelphia, PA: F.A. Davis, 1983.
5. Mark E. Brecher, MD et al. Technical Manual 15th Edition, Bethesda, MA: AABB, 2005.

Key: Underline = Addition of changes    ◀ = Deletion of text